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(54) **PROCESS FOR THE SELECTIVE PREPARATION OF PARTIALLY ACYLATED DERIVATIVES OF MONOSACCHARIDES AND POLYOLS**

VERFARHREN ZUR HERSTELLUNG VON TEILWEISE ACYLIERTEN DERIVATIVEN VON MONOSACCHARIDEN UND POLYOLEN

PROCEDE DE PREPARATION SELECTIVE DE DERIVES PARTIELLEMENT ACYLES DE MONOSACCHARIDES ET DE POLYOLS

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Description

Sector of the technique

[0001] The invention belongs to the alimentary, pharmaceutical, cosmetic and chemical sectors. The products obtained are excellent biodegradable and biocompatible nonionic tensioactive agents which are used as additives for their emulsifying, oily, thickening and moisturising properties.

State of the technique

[0002] The employment of enzymes in organic media is an attractive alternative to classical organic synthesis. The main drawbacks of conventional chemical techniques are their high energy consumption (high temperatures and pressures), the low selectivity of the processes and/or the employment of inorganic catalysts which produce coloured impurities of variable toxicity. In contrast, enzymatic catalysts can achieve selective synthesis of a given product under less extreme pressures and temperatures and are less environmentally toxic.

[0003] Lipases are the hydrolytic enzymes most effective at catalytic processes involving long chain fatty acids. These form covalent bonds with alcohols in media with a low degree of hydration. Because of their versatility and wide availability these make very valuable industrial biocatalysts. Besides, lipases are biodegradable. When several hydroxyl groups are present in the same molecule, because of their extreme selectivity lipases can replace long synthetic pathways (with phases of protection /exposure) with one enzymatic step. This increases yields and reduces the cost of the process itself and also that associated with treatment of the waste products [Biocatalysts for industry (1991) PLenum Press, N.Y. Ed. J. Dordick] compared to alternative chemical processes. Moreover, the chemo- and enantioselectivity of lipases can give rise to highly competitive synthetic processes.

[0004] More than 80% of the world market of tensioactive agents are molecules formed by condensation between polyols or monosaccharides and long chain fatty acids (glycerides, spanes, tweenes etc.). Esterified sorbitan derivatives with long chain fatty acids are biocompatible glycolipid surfactants commonly used in a variety of industries. The commercial name for esterified sorbitan derivatives is Spanes. These are highly lipophilic and are precursors of Tweenes. At present, Tweenes and Spanes are prepared industrially by non-enzymatic procedures at 180-150ºC. Gluco and glycolipidic surfactants are additives used in cosmetic and pharmaceutical products for their different properties (moisturizing and lubricative capacities etc.). The employment of many of these emulsifiers, which are themselves biocompatible, for certain applications (e.g. in the alimentary sector) is often problematic. These problems are related with the conventional chemical method used in their preparation. The presence of certain synthetic agents in the chemical processes used to manufacture or purify the additive constitute one of the main drawbacks of the preparation methods used. Selective obtainment of emulsifiers by enzymes can solve this problem. European and American legislation consider substances produced or modified by enzymes as natural products.

[0005] The enzymatic production of glucolipidic tensioagents derived from monosaccharides and polyols has been studied in the literature although only low yields were obtained [Khaled, N., Montet, D. Pina, M. and Graille, J. Biotech. Let., 13, 167-172 (1991) and Schlotterbeck, A. Lang, S., Wray, V. and Wagner, F. Biotechnol. Lett. 15, 61-64 (1993)] and/or these require preliminary non-enzymatic steps [Fregapane, G., Sarney, D.B. and Vulfson, E.N. Enz. Microb. Technol.13, 796-800 (1991) and Bjorklong, F., Godtfredsen, S.E. and Kirk, O.J.Am. Soc. chem. Commun. 934-935 (1989)]. One enzymatic method based on an aqueous mixture of reagents at 20-60°C [DE-B-3430944 (SEINO)] and the later improved version of the process using high pressure water evaporation [JP-A-62195292] have been disclosed. In both cases the yields obtained are relatively low due to displacement of the thermodynamic equilibrium of the aqueous process towards hydrolysis. The sugars recommended in these patents are glucose, saccharose, raffinose, dextrin, mannan, cellulose, sorbitol and xylitol. Yields have been increased using almost anhydrous organic media and increasing the solubility of the starting sugar in the medium by previous chemical modification. This was also the aim of a number of international patents. A further esterification process, JP-A-63-112993, published on the 18th of May 1988 includes a preliminary step that consists of acetylating the sugar, whereas according to EP-A-0334498 the glucoside is alkylated before carrying out the enzymatic acylation. The processes described in the aforecited prior art literature all include a previous non-enzymatic step to decrease the polarity of the substrate followed by enzymatic processes with high yields. However, the tensioactive molecules obtained are more complex than those obtained with the previous methods.

[0006] EP-A-0506159 and EP-A-0274798 disclose processes for esterification of carboxylic acids with alcohols in the presence of lipases. In the case of EP-A-0506159 a mixture of the alcohol and the acid are contacted with an esterification enzyme and the water formed during the reaction is eliminated by pervaporation. The solvent is selected to ensure that the reactants have mutual solubility and enzyme catalyst activity is not impared. In the case of EP-A-0274798 the acid and alcohol are contacted with lipase and the alcohol is added at a rate that the concentration thereof is maintained below a specific value.

Brief Description of the Invention

[0007] This invention as claimed consists of a process for selective preparation of partially acylated monosaccharide and linear polyol derivatives which are esterified only in respect of their primary hydroxyls, the process comprising the steps of preparing a reaction mixture of a linear polyol or a monosaccharide with the exception of galactose, a fatty acid or a mixture of fatty acids, and a solvent, and contacting the reaction mixture with a lipase, and continuously removing water formed during the process according claim 1.

Detailed description of the invention

[0008] The invention refers to the highly selective enzymatic production of monosaccharides and polyols being mono and/or disubstituted with fatty acids. The procedure is based on a simple mixture of polyol or monosaccharide with the fatty acid, the addition of solvent and the use of lipases that permit the highly selective obtainment of these products without catalysts or toxic agents. The water produced during the process must be continually removed throughout the process.

[0009] The tensioactive agents synthesized present a higher biodegradability than ethoxylated alkyl phenols commonly used in washing powders etc.

[0010] Moreover, these enzymatic reactions permit the recovery and reassessment of the waste products. Lipases can act with pure fatty acids and also with mixtures of these derived from natural oils and fats (olive, coconut or palm oil etc.) The European Union produces large surpluses of oils and fats. Minimization of the waste products and the recovery of byproducts (oils and fats) and their industrial reassessment and recycling is one of the priority objectives in Europe and elsewhere.

[0011] The chemical procedures currently used for the industrial preparation of these products involve high energy consumption and the corresponding installations for conditions of high pressure and temperature. Because of the low selectivity of these chemical procedures [US-A-2759 922], unlike enzymatic processes these chemical procedures can not obtain products with a specific and constant structure. Previously patented enzymatic procedures [DE-B-3439944 (SEINO) and JP-A-62195292] which use aqueous medium, achieve very low yields, limiting their industrial use. The present enzymatic process can achieve almost quantitative yields by direct esterification of the polyol and/or monosaccharide in acetone. Our invention can directly use the polyol or sugar without the prior chemical modification described in JP-A-63-112993
and EP-A-0334498 which obtain different final products than those obtained here. In both these procedures the previous non-enzymatic step and also the possible use of solvents with a high boiling point make the patented processes more expensive than the present procedure. The present method preferentially uses cheaper solvents of easy elimination and low toxicity.

[0012] The invention is applicable to linear polyols such as sorbitol. Also, monosaccharides such as glucose, fructose, mannose etc. can be selectively transformed. The reaction with galactose is less selective and results in a greater mixture of mono and diesters.

[0013] With the patented procedure, different fatty acids of variable chain length, preferably from C8 to C22 can be used. The acid chain can be saturated or unsaturated, linear or branched (i.e. octanoic acid, hexadecanoic acid, oleic acid etc.). Also, a mixture of fatty acids can be used (i.e. industrial acids from coconut, soya, corn, cotton seeds, etc.). Direct use of ethylic esters of fatty acids is much less effective in these processes.

[0014] High temperatures are not required milder temperatures (0-60ºC) are used.

[0015] The process is catalyzed by lipolytic enzymes of yeast or animal, vegetable, bacterial or fungal origin. This can be in a native form, immobilized in an inert support and/or modified chemically or can be obtained by genetic engineering. Thus, high yields have been obtained with immobilized lipases of *Candida antartica* (Novozim 435) and *Mucor miehie* (Lipazyme IM) prepared by Novo Industria A/S, with Novozim 435 proving very effective.

[0016] The process is carried out such that selective esterification of primary hydroxyls of the polyol or monosaccharide is achieved without alteration of secondary hydroxyls thereof.

[0017] The water in the reaction mixture is maintained between 0.001 and 5% wt/wt.

[0018] The solvent is acetone.

[0019] The fatty acid content is above 100 mM and has a molar excess of 1.5 times that of the sugar.

[0020] The process should be carried out in a continuously agitated tank. The amount of enzyme required depends on the specific activity of the enzyme preparation used.

[0021] The invention will be specifically described hereinafter by the following examples:

Example 1

Synthesis of glucose 6-monolaurate

Example A

[0022] 2 ml of acetone were added to the reactor which contained 100 mg of Novozim 435, 60 mg of glucose and 200 mg of lauric acid. The reaction was carried out under mild continual agitation at 40ºC and water was continually removed from the reaction medium. 121 mg of glucose 6-monolaurate were obtained after 3 days of reaction corresponding to a yield of 99%.

Example B

[0023] 8g of Novozim 435, 5g of glucose and 16.7g of

lauric acid were added to 160 ml of acetone. After 5 days of mild agitation at 40ºC with constant removal of water from the reaction mixture, 9.6g of glucose 6-monolaurate (98% yield) were obtained.

Example C

[0024] 80 g of Novozim 435,50 g of glucose and 167g of lauric acid were added to 1.61 of acetone. After 6 days of mild agitation at 40ºC and with continuous removal of water from the reaction medium 90 g of glucose 6-mono-laurate were obtained (90% yield).

Example 2

Reaction between sorbitol and the fatty acids derived from hydrolysis of olive oil

[0025] 60 mg of sorbitol, 480 mg of a mixture of fatty acids and 100 mg of Novozim 435 were added to 2 ml of acetone. The reaction was carried out under continuous mild agitation and water was continually removed from the medium that was maintained at 40ºC for 6h and later at 10ºC. 234 mg of 1,6 sorbitol diester and 8 mg of monoester corresponding to yields of 94% and 2% respectively were obtained.

Example 3

Reaction between mannose and lauric acid

[0026] This reaction used 60 mg of mannose and 200 mg of lauric acid in 2 ml of acetone in the presence of 100 mg of Novozim 435, the reaction mixture was maintained at 60ºC and under continuous mild agitation and water was continually removed from the reaction mixture. 107 mg of monoester and 18 mg of diester were obtained after 15 hours. The yields were 90% and 10%, respectively.

Claims

1. A process for selective preparation of partially acylated monosaccharide and linear polyol derivatives which are esterified only in respect of their primary hydroxyls, the process comprising the steps of preparing a reaction mixture of a linear polyol or a monosaccharide with the exception of galactose, a fatty acid or a mixture of fatty acids, and a solvent, and contacting the reaction mixture with a lipase, and continuously removing water formed during the process wherein

the process is carried out at a reaction temperature between 0º and 60ºC;
the water in the reaction mixture is maintained between 0.001 and 5% wt/wt;
the fatty acid or mixture of fatty acids, is added in a concentration higher than 100mM and in a molar excess of at least 1.5 fold, in respect of the polyol or the monosaccharide,
the process is carried out such that selective esterification of primary hydroxyls of the polyol or monosaccharide is achieved without alteration of secondary hydroxyls thereof;
the solvent is acetone.

2. A process according to claim 1, wherein the lipase is selected from the group of immobilized lipases of *Candida antartica* and *Mucor miehie*.

3. A process according to claim 1, wherein the mixture of fatty acids comprises C_8 - C_{22} fatty acids having a linear or branched, saturated or insaturated chain.

4. A process according to claim 1, wherein the-fatty acid is lauric acid.

5. A process according to claim 1, wherein the fatty acid is selected from the group consisting of octanoic acid, hexadecanoic acid and oleic acid.

6. A process according to claim 1, wherein the fatty acid is selected from the group consisting of industrial acids from coconut, soya and cotton seeds.

7. A process according to claim 3, wherein the mixture comprises at least one of octanoic acid, hexadecanoic acid and oleic acid.

8. A process according to claim 1, wherein the mixture comprises at least one industrial acid from the group consisting of coconut, soya and cotton seeds.

9. A process according to claim 1, wherein the reaction temperature is 0-40ºC.

10. A process according to claim 9, wherein the reaction temperature is 40ºC.

11. A process according to claim 1, wherein the monosaccharide is selected from the group consisting of glucose, mannose and fructose.

12. A process according to claim 1, wherein the linear polyol is sorbitol.

13. A process according to claim 1, wherein the polyol is sorbitol, a mixture of fatty acids obtained by hydrolysis of olive oil is used, and the reaction mixture is maintained at a temperature of 40ºC for 6h and thereafter maintained at 10ºC.

Patentansprüche

1. Verfahren zur selektiven Herstellung von teilweise acylierten Monosaccharid- und linearen Polyol-Derivaten, welche nur im Hinblick auf ihre primären Hydroxyle verestert sind, wobei das Verfahren die folgenden Schritte umfasst: das Herstellen eines Reaktionsgemisches aus einem linearen Polyol oder einem Monosaccharid mit Ausnahme von Galactose, einer Fettsäure oder einem Fettsäurengemisch und einem Lösungsmittel, und das Inkontaktbringen des Reaktionsgemisches mit einer Lipase, und das kontinuierliche Entfernen von Wasser, das während des Verfahrens gebildet wird, wobei

   das Verfahren bei einer Reaktionstemperatur zwischen 0° und 60°C durchgeführt wird;

   das Wasser in dem Reaktionsgemisch zwischen 0,001 und 5 % Gew./Gew. gehalten wird;

   die Fettsäure oder das Fettsäurengemisch in einer Konzentration von mehr als 100 mM und in einem mindestens 1,5fachen molaren Überschuss, bezogen auf das Polyol oder das Monosaccharid, zugegeben wird,

   das Verfahren so durchgeführt wird, dass eine selektive Veresterung von primären Hydroxylen des Polyols oder Monosaccharids ohne Veränderung der sekundären Hydroxyle davon erreicht wird;

   das Lösungsmittel Aceton ist.

2. Verfahren nach Anspruch 1, wobei die Lipase ausgewählt ist aus der Gruppe von immobilisierten Lipasen von *Candida antartica* und *Mucor miehie*.

3. Verfahren nach Anspruch 1, wobei das Fettsäurengemisch C_8-C_{22}-Fettsäuren mit einer linearen oder verzweigten, gesättigten oder ungesättigten Kette umfasst.

4. Verfahren nach Anspruch 1, wobei die Fettsäure Laurinsäure ist.

5. Verfahren nach Anspruch 1, wobei die Fettsäure ausgewählt ist aus der Gruppe, bestehend aus Octansäure, Hexadecansäure und Ölsäure.

6. Verfahren nach Anspruch 1, wobei die Fettsäure ausgewählt ist aus der Gruppe bestehend aus Industriesäuren aus Kokosnuss, Soja und Baumwollsamen.

7. Verfahren nach Anspruch 3, wobei das Gemisch mindestens eine der folgenden Säuren umfasst: Octansäure, Hexadecansäure und Ölsäure.

8. Verfahren nach Anspruch 1, wobei das Gemisch mindestens eine Industriesäure aus der Gruppe bestehend aus Kokosnuss, Soja und Baumwollsamen umfasst.

9. Verfahren nach Anspruch 1, wobei die Reaktionstemperatur 0-40°C beträgt.

10. Verfahren nach Anspruch 9, wobei die Reaktionstemperatur 40°C beträgt.

11. Verfahren nach Anspruch 1, wobei das Monosaccharid ausgewählt ist aus der Gruppe bestehend aus Glucose, Mannose und Fructose.

12. Verfahren nach Anspruch 1, wobei das lineare Polyol Sorbitol ist.

13. Verfahren nach Anspruch 1, wobei das Polyol Sorbitol ist, ein Fettsäurengemisch, das durch Hydrolyse von Olivenöl erhalten wird, verwendet wird und das Reaktionsgemisch 6 h bei einer Temperatur von 40°C gehalten wird und danach bei 10°C gehalten wird.

Revendications

1. Procédé de préparation sélective de dérivés partiellement acylés de monosaccharides et de polyols linéaires qui sont estérifiés seulement du point de vue de leurs groupes hydroxyle primaires, le procédé comprenant les étapes consistant à préparer un mélange réactionnel d'un polyol linéaire ou d'un monosaccharide à l'exception du galactose, d'un acide gras ou un mélange d'acides gras et d'un solvant, et à mettre en contact le mélange réactionnel avec une lipase et à éliminer continuellement l'eau formée au cours du procédé dans lequel

   le procédé est effectué à une température de réaction comprise entre 0°C et 60°C ;
   l'eau dans le mélange réactionnel est maintenue entre 0,001 et 5% p/p ;
   l'acide gras ou le mélange d'acides gras est ajouté en une concentration supérieure à 100 mM et en un excès molaire égal à au moins 1,5 fois, du point de vue du polyol ou du monosaccharide ;
   le procédé est effectué de telle sorte que l'estérification sélective des groupes hydroxyle primaires du polyol ou du monosaccharide soit obtenue sans altération de leurs groupes hydroxyle secondaires ;
   le solvant est l'acétone.

2. Procédé selon la revendication 1, dans lequel la lipase est sélectionnée parmi le groupe de lipases immobilisées de *Candida antartica* et *Mucor miehie.*

3. Procédé selon la revendication 1, dans lequel le mélange d'acides gras comprend des acides gras en C_8-C_{22} ayant une chaîne saturée ou insaturée, linéaire ou ramifiée.

4. Procédé selon la revendication 1, dans lequel l'acide gras est l'acide laurique.

5. Procédé selon la revendication 1, dans lequel l'acide gras est sélectionné parmi le groupe constitué par l'acide octanoïque, l'acide hexadécanoïque et l'acide oléique.

6. Procédé selon la revendication 1, dans lequel l'acide gras est sélectionné parmi le groupe constitué par des acides industriels provenant de graines de coco, de soja et de coton.

7. Procédé selon la revendication 3, dans lequel le mélange comprend au moins un acide parmi l'acide octanoïque, l'acide hexadécanoïque et l'acide oléique.

8. Procédé selon la revendication 1, dans lequel le mélange comprend au moins un acide industriel parmi le groupe constitué par les graines de coco, de soja et de coton.

9. Procédé selon la revendication 1, dans lequel la température de la réaction varie de 0 à 40°C.

10. Procédé selon la revendication 9, dans lequel la température de la réaction est de 40°C.

11. Procédé selon la revendication 1, dans lequel le monosaccharide est sélectionné parmi le groupe constitué par le glucose, le mannose et le fructose.

12. Procédé selon la revendication 1, dans lequel le polyol linéaire est le sorbitol.

13. Procédé selon la revendication 1, dans lequel le polyol est le sorbitol, un mélange d'acides gras obtenu par hydrolyse de l'huile d'olive est utilisé et le mélange réactionnel est maintenu à une température de 40°C pendant 6 h et maintenu à 10°C par la suite.